

BCH 322

[Experimental Enzymology]

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Course Description

- A set of special experiments designed to study parameters of enzyme activity, activation & inhibition, and characterization of enzymes.

	Title of the Experiments
1	Some Factors Affecting Polyphenol Oxidase Activity
2	Methods of Enzyme Assay
3	Purification of Acid Phosphatase from Wheat Germ
4	The Effect of Incubation Time on the Rate of an Enzyme Catalyzed Reaction
5	The Effects of Temperature on the Rate of an Enzyme Catalyzed Reaction
6	The Effects of Enzyme Concentration on the Rate of an Enzyme Catalyzed Reaction
7	The Effects of pH on the Rate of an Enzyme Catalyzed Reaction
8	The Effects of Substrate Concentration on the Rate of an Enzyme Catalyzed Reaction
9	The Inhibition of Acid Phosphatase by Inorganic Phosphate

Mark Distribution

	Marks	
Conducting the experiment	5 Marks	
Report	15 Marks	
Quiz	15 Marks	
Midterm (25 Marks)	Practical	15 Marks
	Theoretical	10 Marks
Final (40 Marks)	Practical	25 Marks
	Theoretical	15 Marks
Total	100 Marks	

Date of midterm exam:

/ /1438 H - / /2016

Date of final exam:

21 / 3 /1438 H – 20 /12 /2016

How to write a scientific report?

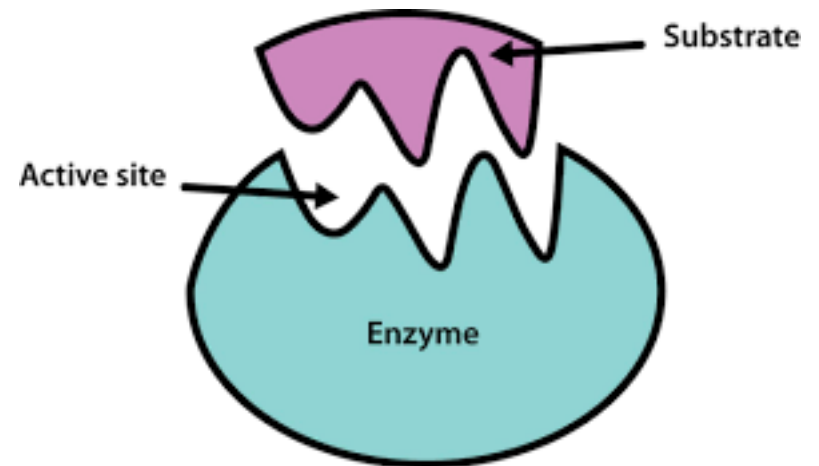
- The laboratory reports should contain the following sections:
 - Title
 - Objectives
 - Brief Introduction [**Theoretical background information**]
 - Materials and Methods [**As seen in the lab sheet**]
 - Results [**Tables, Graphs and/or Calculations**]
 - Discussion
 - In this section you are required to describe of what happened in the experiment **(Principle)**, explain your results and make conclusions by comparing your results to expected values (calculated or from the literature).
 - Even if you obtained unexpected results, the discussion section is the section to justify or explain the reasons why you have obtained such results.
 - Questions

Safety in the Lab



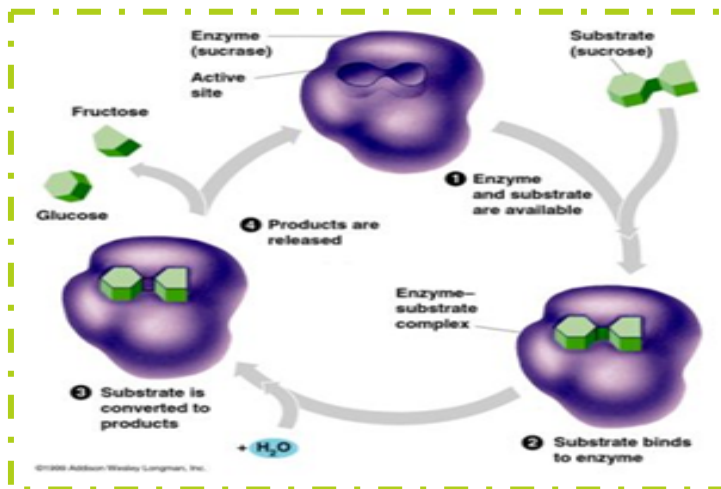
- You must wear a lab coat and hand gloves.
- Open toed shoes must not be worn because they cannot protect you against chemical spills.
- Long hair should be tied back to avoid any interference with the experiment.
- In case of acid or base contact with your skin, wash it with large amount of clean, cold water and inform the instructor immediately.
- Do not handle broken glassware with your bare hands.
- Do not eat, drink, or chew gum in the laboratory.
- Do not depart from the lab leaving an experiment unattended. If you need to leave the lab you must inform your instructor before leaving the lab.
- You must wash your hands with soap after finishing the experiment.

Introduction to enzymology



What are Enzymes and its function?

- Enzymes (E) are biological molecules that increase the rates [catalyze] of biochemical reactions *without being consumed*.
- Most catalyzed reactions are **reversible**
- Nearly all known enzymes are **proteins** in nature with the exception of certain RNA molecules called *ribozymes*.

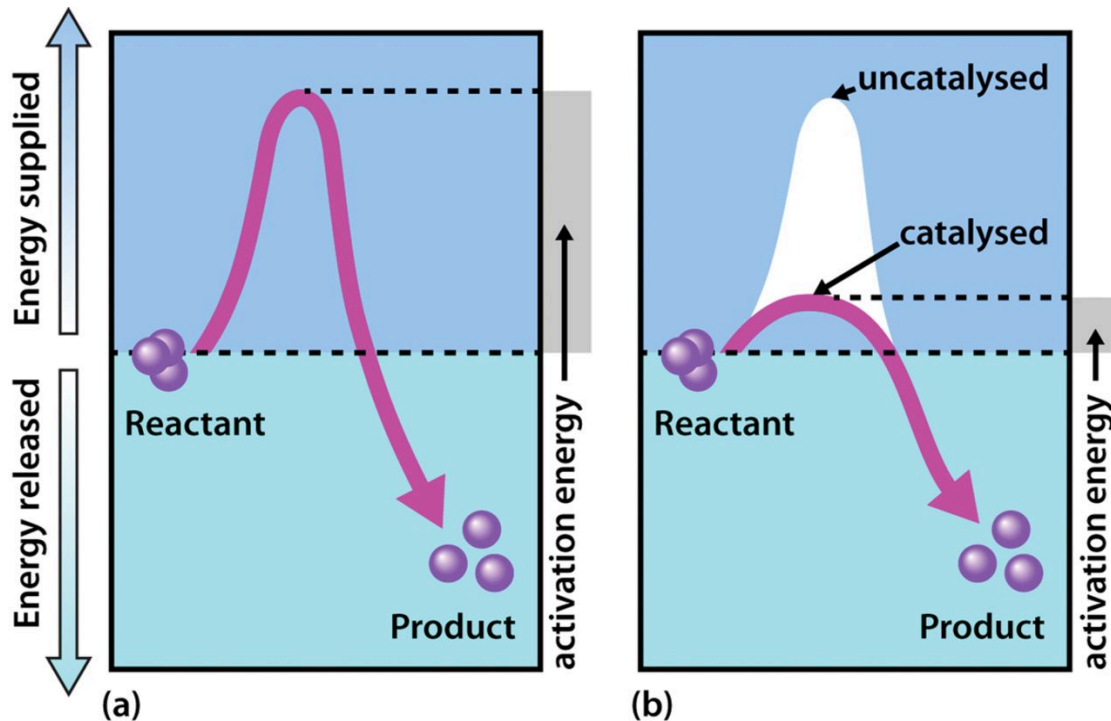


Copy this link to watch

http://highered.mheducation.com/sites/0072495855/student_view0/chapter2/animation_how_enzymes_work.html

How can enzyme increase the rate of a biochemical reactions?

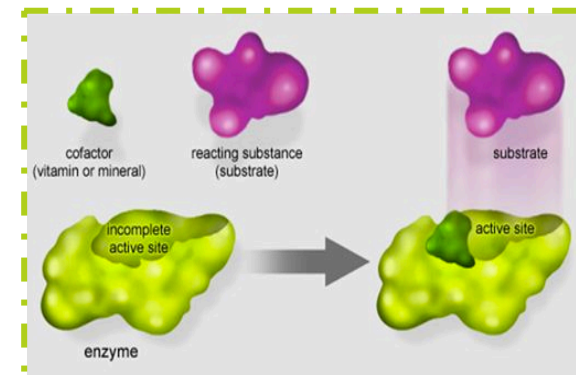
1. Lowering the activation energy
2. Reducing the chance in the collisions of molecules or ions



Activation energy : Is the energy that must be overcome in order for a chemical reaction to occur, given in units of **kilojoules/mole**.

Enzyme Terminology

- Substrate (S): a molecule upon which an enzyme acts on.
- Active site: The specific site on an enzyme where the S binds and catalysis occur.
- E-S complex: an intermediate formed when the substrate molecule binds to the active site of the enzyme.
- Product (P): a substance produced as a result of the reactions.
- Cofactors: Small molecules that binds with some E and are necessary for their function.
Could be either:
 - Inorganic** - metal ions such as Zn^{+2} , Cu^{+2} , or Mg^{+2}
 - Organic (Coenzymes)** – Mostly vitamins and their derivatives such as NAD [derived from niacin (vit B3)]

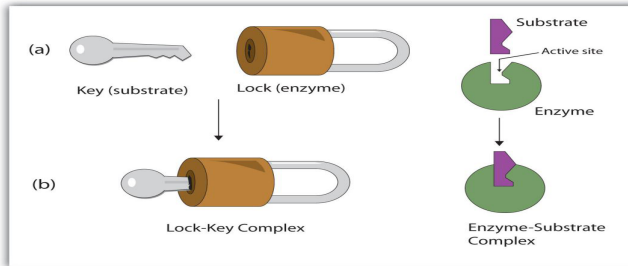


Turnover number

- It is the total number of substrate molecules that an enzyme can convert to product per minute, when the enzyme is fully saturated with substrate.
- It varies from enzyme to another.
- Many enzymes have a high turnover number. For example, **catalase has a turnover number of 5 million per minute.**
- Thus enzymes are generally effective in relatively minute concentrations in the living cell.

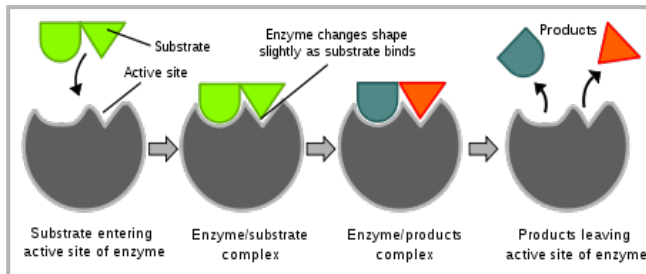
How can a substrate bind to the Enzyme?

The Key and lock hypothesis



Suggests that both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another

Induced fit model



Suggests that enzymes are flexible structures, where the the amino acid side chains which make up the active site are continually reshaped by interactions with the substrate.



Notes in practical work

In this course enzymes will be extracted from cells and the enzyme catalyzed reactions will be studied in a test tube.

In any experiment you should know:

- The aim and what you are doing while working
- You must know why and what are the importance of the chemicals that you are adding during the experiment
- You should be accurate

Instruments used in this course are:



Spectrophotometer



Waterbath



Pipette



Micropipette